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UTILIZATION OF ^{3}H-THYMIDINE TRIPHOSPHATE BY DEVELOPING STAGES OF *PECTINARIA GOULDII*

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While earlier experiments (Tweedell, 1966) had demonstrated that ^{3}H-thymidine (^{3}H-TdR) was incorporated into nuclei of ovarian preoocytes in the polychaete *Pectinaria gouldii*, no uptake was observed in any oocyte stage during the vegetative period of oocyte growth and differentiation in the coelom. Nuclear DNA synthesis is apparently absent throughout the entire period of oogenesis until after fertilization. The failure of thymidine uptake during oocyte development might be attributed to the absence of thymidine kinase, other phosphorylating enzymes or the necessity of having a complete pool of DNA nucleosides. Utilization of ^{3}H-thymidine triphosphate (^{3}H-TTP) as an indicator of DNA synthesis seemed justified since uptake of deoxyribonucleoside triphosphates has been observed in membrane altered *E. coli* cells (Buttin and Kornberg, 1966; Moses and Richardson, 1970) and in growing cells of *Stentor coeruleus* (de Terra, 1967). Uptake of TTP *in vitro* by isolated nuclei of rat liver (Kaufman, Grisham and Stenstrom, 1972), rat thymus (Lagunoff, 1969) and HeLa cells (Friedman and Mueller, 1968) has also been obtained. Possible use of TTP by the developing embryo also seemed likely since isolated nuclei of embryonic, regenerating and neoplastic tissues showed a marked increase of TTP uptake above that found in normal rat nuclei (Lynch, Brown, Umeda, Langreth and Liebermann, 1970).

The potential incorporation of TTP into mitochondria of the oocyte of embryo was predicated by similar uptake *in vitro* by isolated mitochondria (Parsons and Simpson, 1967). To investigate each of these possibilities, developing stages of *Pectinaria* from the preoocyte stage through gastrulation were exposed to ^{3}H-TTP or ^{3}H-TdR alone or in combination with their respective complementary unlabeled nucleotides or nucleosides.

Materials and Methods

Isotope presentation

Labeled nucleic acid precursors utilized were thymidine-methyl-H^3(TdR) (6.7 Ci/mM) in a concentration of 0.018 mg/ml and *d*-thymidine-methyl-H^3-5′ triphosphate (^{3}H-TTP) as a tetralithium salt (2.8 Ci/mM; Schwartz) or as a tetrasodium salt [15.7 Ci/mM (New England Nuclear)] at a concentration of 0.009 mg/ml. Experiments were repeated over a period of three summers with three separate lots of radioactive precursors.

In vivo *experiments*

Isotopes were diluted with sterile distilled water and injected through the cephalic placque into the coelomic cavity of adult animals after anesthesia with

50% ethanol (Tweedell, 1966). From 50 to 10 μCi of ^{3}H-TTP in 0.1 ml quantities were injected depending upon the volume of coelomic fluid. The average volume of coelomic fluid from large mature animals was determined to be 0.3 ml after cell removal. After returning the animals to their sand tests they were placed in running sea water for the pulse duration.

Nine adult females were injected with 5 to 10 μCi/animal of ^{3}H-TTP only and pulsed for periods of 0.5, 1, 2 and 4 hours. Several other adults received ^{3}H-TTP in the presence of the three unlabeled deoxynucleoside triphosphates for 0.5 to 2 hours, and one had an exposure of six days.

Postshedding

Oocytes from females were shed into sea water and germinal vesicle breakdown occurred in mature oocytes 15–20 min afterwards. The oocytes were washed and previously shed spermatozoa were added (6 drops/dish) 30 min after germinal vesicle breakdown. Fifteen minutes later the fertilized eggs were washed to remove excess spermatozoa and transferred into Millipore-filtered sea water. Aliquots of either oocytes, fertilized eggs or developmental stages were collected by light centrifugation and placed in 10 ml volumes of pasteurized sea water with 2.5 to 5 μCi/ml of either ^{3}H-TdR or ^{3}H-TTP. Either precursor was diluted with sterile distilled water and introduced as 0.1 ml quantities to give a final concentration of 0.0015 mM.

Unlabeled precursors

Samples of cells were also incubated with unlabeled nucleosides or nucleotides with the corresponding labeled nucleic acid precursor. Unlabeled *d*-adenosine, *d*-guanosine (0.01 mM) and *d*-cytosine (0.03 mM) were added with ^{3}H-thymidine to 10 ml of pasteurized sea water or up to 0.16 mM *in vivo*. In practice, they were contained in the sterile distilled water used to dilute the isotope. Unlabeled deoxynucleoside triphosphates, *d*-adenosine triphosphate (0.005 mM), *d*-guanosine triphosphate (0.005 mM) and *d*-cytosine triphosphate (0.005 mM) were added to ^{3}H-TTP in the incubation mixture.

Histology and autoradiography

Intact ovaries and oocytes after isotope exposure were preserved *in situ* with Kahle's fixative. The anterior one-third of the animal was embedded in methacrylate plastic and sectioned at 1 or 2 μ on a Sorvall J-B4 microtome. Eggs and embryos were also harvested after *in vivo* or external exposure to isotopes by swirling them into Kahle's fixative, followed by embedding in paraffin and sectioning at 5 μ. Selected sections of ovaries or eggs were treated with DNAase I (Sigma) prior to autoradiography in a concentration of 0.1 mg/ml in 0.05 M veronal acetate buffer, pH 6.8 with 0.003 M $MgCl_2$. Incubation was at 37° C for two hours.

Slides were dipped in Ilford L4 (1:1) or Kodak NTB2 emulsions. They were incubated at 4° C for two to three weeks, developed in D-72 for 3 min at 14° C, rinsed in water and fixed for 5 min. After washing they were stained in Jenner-Giemsa solution, differentiated in 0.1 N HCl and mounted in Permount.

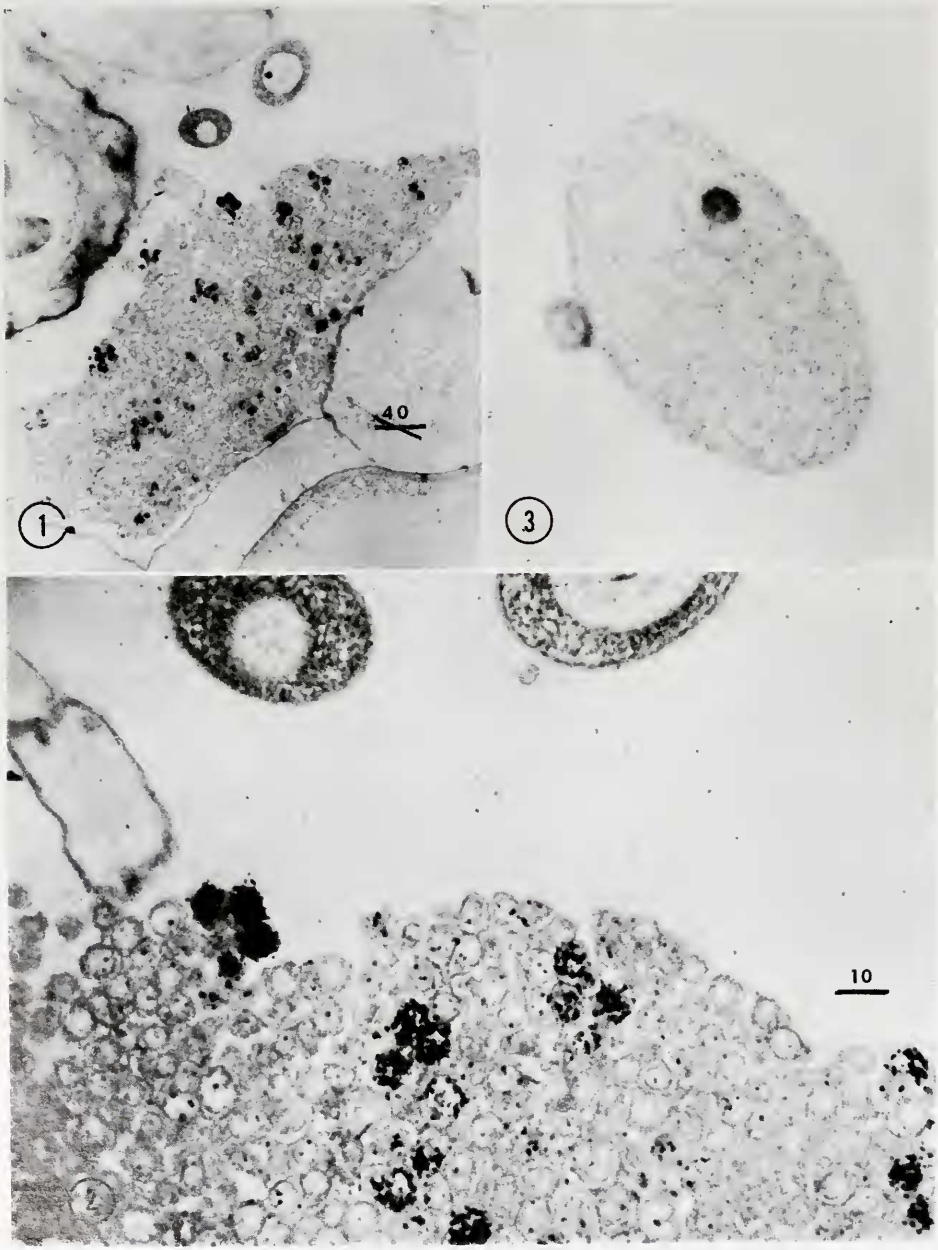

FIGURE 1. Scattered groups of preoocytes in the ovary after 1 hour exposure to ^{3}H-TTP; scale is 40 microns.

FIGURE 2. Distribution of ^{3}H-TTP uptake over nuclei of preoocytes of ovary. Some heavily labeled cells are seen budding from the edge of the ovary; scale is 10 microns.

FIGURE 3. Section through the trophozoite stage of a coelomic gregarine, *Urospora* sp. showing intense cytoplasmic labeling after exposure to ^{3}H-TTP for 1 hour. Note that the nucleus and an attached oocyte are unlabeled.

Results

Since previous investigations of TTP incorporation *in vitro* had indicated the necessity of having all deoxyriboside triphosphates present, the concentration toxicity of either the unlabeled nucleotides or unlabeled nucleosides was determined on the development of *Pectinaria* eggs. Eggs were fertilized with preshed and washed sperm in 0.01 mM, 0.1 mM, and 1 mM concentrations of *d*-adenosine triphosphate (ATP), *d*-guanosine triphosphate (GTP) and *d*-cytidine triphosphate (CTP), prepared in pasteurized sea water prior to the introduction of the eggs. Equal volumes of fertilized eggs were added to 10 ml of each nucleotide dilution. After 22 hours all dilutions contained swimming gastrulae and larvae which indicated there was no toxic effect at these concentrations. Similar tests at the same dilutions were done on the four deoxynucleosides and they also failed to elicit a concentration toxicity.

Exposure to ^{3}H-TTP

The ovary. Normally, the ovary contains a mixed population of oogonial cells and preoocytes. In all cases after pulsing with ^{3}H-TTP a heavy nuclear label appeared over pairs or groups of cells scattered throughout the ovary; after four hours the label became very dense (Fig. 1). These cells, measuring 4–5 μ in diameter, have a very thin peripheral rim of cytoplasm, consequently the entire surface of the preoocytes sometimes appeared densely covered with silver grains. In thin 1 μ sections, when uptake was less dense, it was clearly located over the nucleus. Very heavily labeled cells were sometimes seen budding from the edge of the ovary into the coelom (Fig. 2), and this is the only example of free oocytes being labeled.

Both the distribution of silver grains and the amount of uptake appeared to be unaffected by the addition of complementary unlabeled nucleotides to ^{3}H-TTP. After treatment of the ovarian sections with DNAase I (0.1 mg/ml) for two hours prior to dipping, radioactivity disappeared from residual oocyte packets and host connective tissue. The nuclear label over the intact ovarian cells was quantitatively reduced but not completely eliminated.

Coelomic oocytes. Developmental stages of the primary oocyte labeled *in vivo* for 0.5, 1, 2 or 4 hours were examined both *in situ* within the coelomic cavity before germinal vesicle breakdown and after being induced to shed from an injected animal. The latter cells undergo germinal vesicle breakdown prematurely and progress into the first maturation division. Normally oocytes bud from the ovary into the coelomic fluid as small cell packets. After a period of cell growth within the packet, the cells separate and begin a phase of vegetative growth as separate, single oocytes until the mature oocytes are selectively taken into the nephromixia (Tweedell, 1966).

Sections taken through the coelom indicated that the full size range of oocyte packets remained unlabeled even after pulsing up to four hours. Single vegetative oocytes from the smallest individual oocytes to the largest mature primary oocyte also remained completely unlabeled. Application of either ^{3}H-TTP alone or with ATP, GTP and CTP gave the same results. After a six day exposure with 10 μCi of ^{3}H-TTP and the three unlabeled nucleotides, small, medium and fully

mature oocytes were still unlabeled. Oocyte packets were also negative; however, some of the rosette clusters of coelomic cells and amoebocytes showed dense labeling over the entire cell surface. None of the oocyte packets showed any definitive label above the background.

One of the coinhabitants of the coelomic fluid with the developing oocytes is a prominent gregarine protozoan, *Urospora* sp. (Brasil, 1904). Simultaneous exposure of the adult vegetative form (trophozoite) *in vivo* to ^{3}H-dTTP revealed a heavy accumulation of silver grains over the entire cytoplasm (Fig. 3). The large, inactive nucleus was unaffected. None of the surrounding oocytes stages showed any indication of incorporation.

Postshedding exposure of oocytes to ^{3}H-TdR

Previous experiments (Tweedell, 1966) had demonstrated the lack of ^{3}H-TdR utilization by developing oocytes but active incorporation of ^{3}H-uridine; as a reaffirmation, new experiments were performed with ^{3}H-TdR and reinforced with unlabeled nucleosides. The incorporation of thymidine into cell fractions of regenerating liver was shown to be stimulated by a mixture of dAMP, dGMP and dCMP (Bollum, 1958). The presentation of ^{3}H-TdR and the unlabeled nucleosides *in vitro* to newly shed oocytes gave no evidence that the latter compounds implemented the uptake of ^{3}H-TdR by the oocytes.

Oocytes were also exposed to ^{3}H-TTP after being shed from females. Before germinal vesicle breakdown (12–15 min), they were placed in Millipore-filtered sea water with 2.5 to 4 μCi/ml of ^{3}H-TTP. Pulse times varied from 1.5 hours (20° C) to 4 hours at 4° C. No label appeared over the oocyte packets or over any of the solitary oocytes in vegetative growth. Mature oocytes were also refractory either before or after germinal vesicle breakdown. Again, the small coelomic rosette cells developed heavy nuclear labels.

To further detect any cytoplasmic localization of radioactivity the oocytes were allowed to undergo germinal vesicle breakdown before the introduction of ^{3}H-TTP. The cells were then placed into 2.5 to 4.0 μCi/ml of ^{3}H-TTP for 1 hour. After washing and chasing with cold thymidine, the cells were layered over a sucrose cushion and centrifuged at 28,700 $\times$ g at 0° C in order to stratify the cell components (Tweedell, 1962). While good granular stratification was obtained, there was no evidence of isotope uptake in any of the stratified layers.

Precursor uptake of ^{3}H-TdR by developing embryos

As an indication of the baseline activity of DNA synthesis during development, fertilized eggs were pulsed (0.5–1 hour) with ^{3}H-TdR (2.5 μCi/ml) after polar body formation following fertilization and during cleavage, blastula and gastrula stages. Other developing stages (4 cell to gastrula) were exposed to ^{3}H-thymidine with unlabeled *d*-nucleosides: *d*-adenosine, *d*-cytidine and *d*-guanosine. Stages were fixed immediately after pulsing. Heavy nuclear uptake of ^{3}H-TdR was very evident in all of the stages. Nuclei of swimming blastulae and randomly localized nuclei of gastrulae were heavily labeled. The intensity of nuclear uptake was greater in the latter stages at the same concentration and time of exposure. No difference was found in nuclear uptake when unlabeled *d*-nucleosides were used jointly with ^{3}H-Tdr.

^{3}H-TTP utilization

Fertilized eggs from four females were placed in 20 ml of filtered sea water containing ^{3}H-TTP (4 μCi/ml) from 20 min (2nd polar body formation) to 40 min (fusion of pronuclei) after fertilization. After pulsing for 45 min to 1 hour, the embryos were fixed immediately. Heavy nuclear label was found in all embryos of the 2, 4 and 8 cell stage (Fig. 4) after exposure to the labeled nucleotide. Unfertilized eggs (after germinal vesicle breakdown) and immature oocytes again failed to show any absorbance of the isotope.

After fertilization and washing, other embryos in the 4, 8 and 16 cell stages were placed into ^{3}H-TTP (1.5 μCi/ml) with unlabeled *d*-nucleotides (dATP, dCTP and dGTP) for exposure times of 1, 2, 3 and 4.5 hours. Embryos pulsed for 1 hour and then fixed had reached the period of late cleavage; the nuclei of all stages were uniformly and intensely covered with silver grains (Fig. 5). Other embryos were chased with unlabeled dTTP after 1 hour and allowed to develop into early blastulae or swimming blastulae. The nuclei of all blastulae were moderately but evenly labeled after 2.5 hours (Fig. 6). The reduction in labeling probably resulted from dilution of the label by successive nuclear divisions. Free swimming ciliated blastulae were recovered after 4.5 hours and still showed a diluted distribution of label over nuclei. Again, the addition of the unlabeled *d*-nucleotides was not prerequisite, nor did they have any apparent effect on the amount of nuclear label.

Fertilized eggs were also grown to the gastrulae stage (10 hours) and then placed in 2.5 μCi of ^{3}H-TTP plus *d*-nucleotides. After 1 hour they were harvested. Individual labeled nuclei again appeared but their distribution was nonuniform, appearing at random in sections generally toward the interior of the gastrulae (Fig. 7).

^{3}H-TTP and thymidine

The possibility that ^{3}H-TTP was being converted to ^{3}H-TdR before incorporation into the cell was investigated by adding an excess of unlabeled TdR to the labeled ^{3}H-TTP. If conversion to ^{3}H-TdR did occur, the labeled nucleoside would have to compete with the cold thymidine pool and thus the final nuclear label should be greatly reduced. Free swimming blastulae (5 hours old) were collected by centrifugation, washed in sterile sea water and added to 25 μCi of ^{3}H-TTP (0.0015 mmole) in 10 ml of pasteurized sea water. Unlabeled thymidine (0.83 mmole) was added simultaneously. As an additional control, another dish received ^{3}H-TTP plus 0.7 mmole of thymidine, *d*-adenosine, *d*-guanosine and *c*-cytidine. After 1.5 hours, the blastulae were recovered, washed and fixed. The addition of unlabeled thymidine with or without the complete nucleosides had no visible effect on the amount of nuclear uptake. Scattered nuclei still gave strong evidence of ^{3}H-TTP utilization with no apparent reduction in the nuclear label (Fig. 8).

As a measure of the effectiveness of the thymidine dilution effect, parallel experiments were conducted by direct exposure of the ovaries *in situ* to 2.5 μCi/ml of ^{3}H-Tdr (specific activity: 20 Ci/mM) at a concentration of 0.00025 mmole. In a second series the labeled nucleoside was combined with undiluted thymidine at a concentration of 0.125 mmole. Exposure lasted for 0.5 hours.

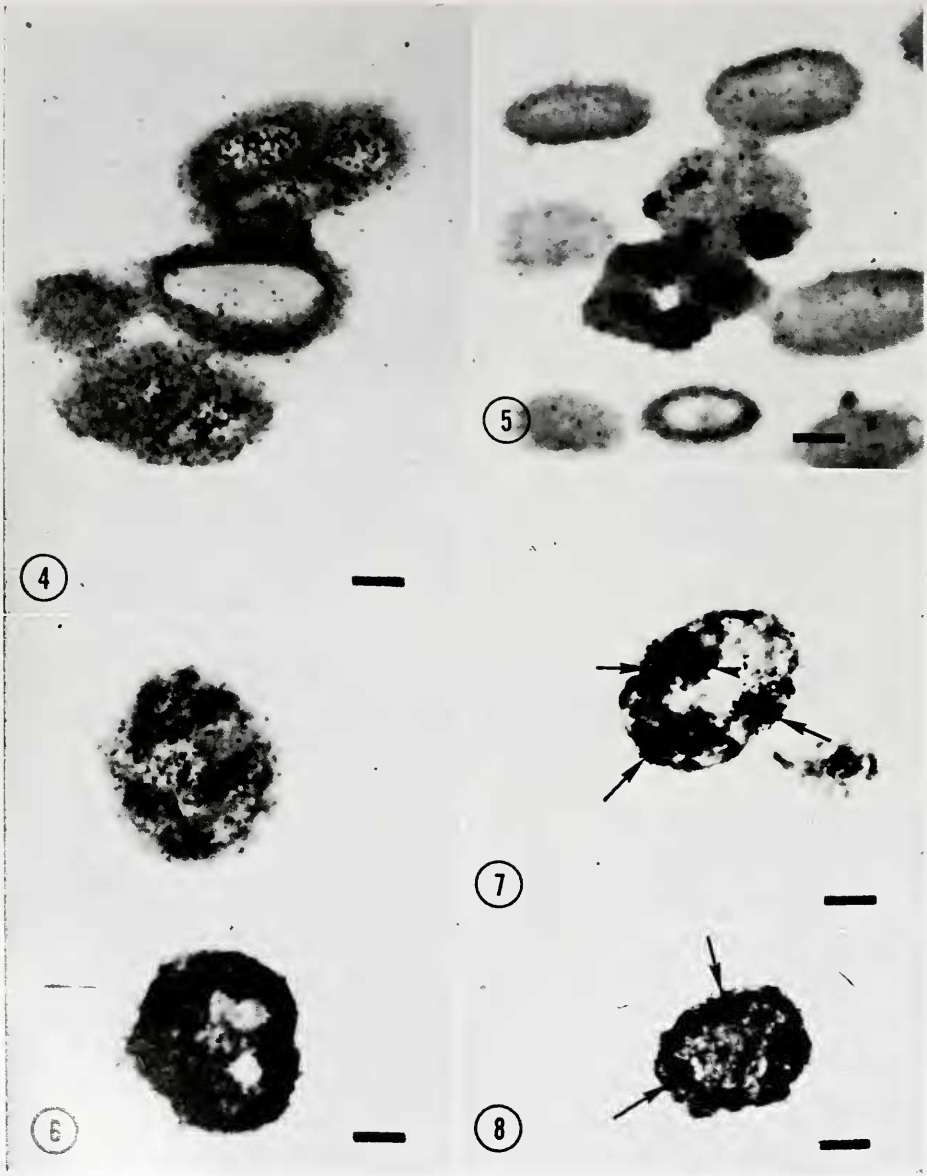

FIGURE 4. Nuclear incorporation of ^{3}H-TTP into 2 and 4 cell embryos after 40 min exposure. Mature oocyte nucleus (center) shows no uptake; scale is 12 microns.

FIGURE 5. Embryos pulsed with ^{3}H-TTP for 1 hour and fixed in late cleavage-early blastula stages, showing heavy nuclear uptake. Mature oocyte is without label; scale is 15 microns.

FIGURE 6. Light label over nuclei of swimming blastulae 2.5 hours after 1 hour pulse chase with ^{3}H-TTP in the 4 cell stage; scale is 14 microns.

FIGURE 7. A gastrula pulsed for 1 hour in ^{3}H-TTP plus *d* nucleotides. Scattered nuclei (arrows) show incorporation; scale is 14 microns.

Label over the ovarian cells with ^{3}H-TdR alone was scattered but the intensity was greatly diminished in the cells exposed to an excess of unlabeled thymidine.

Discussion

The lack of isotope incorporation from ^{3}H-TTP into the nuclei of all coelomic stages of the developing oocytes of *Pectinaria* parallels the earlier finding with ^{3}H-TdR (Tweedell, 1966). Even the addition of all deoxynucleosides of ^{3}H-TdR in the present experiments failed to show thymidine uptake in the oocytes. The results suggest that the oocytes are impermeable to nucleosides and nucleotides or that DNA synthesis is absent or deficient. Yet, earlier experiments showed that ^{3}H-uridine penetrated the oocyte quite readily. Gurdon and Woodland (1968) did find a lack of DNA synthesis after injection of primer DNA and ^{3}H-TdR into growing or full sized oocytes of *Xenopus*. They concluded that DNA polymerase is absent or inactive in the egg cytoplasm at the time of germinal vesicle breakdown. It is also possible that the required kinases are not available during oocyte formation in *Pectinaria*, but oocyte impermeability to TTP is still a possibility.

Simultaneous *in vivo* exposure of ^{3}H-TTP produced cytoplasmic uptake in the parasitic gregarine protozoan *Urospora* found among the developing oocytes in the coelom of the adult *Pectinaria*. It may be significant that the same trophozoit stage pulsed with ^{3}H-TdR failed to show either cytoplasmic or nuclear label. Similar cytoplasmic labeling of *Stentor coeruleus* with ^{3}H-TTP during all stages of the cell growth cycle was observed by de Terra (1967). In the current experiments the cytoplasmic labeling by ^{3}H-TPP may be direct incorporation by mitochondria. Isolated rat liver mitochondria will incorporate ^{14}C-dTTP into DNA (Parsons and Simpson, 1967; Kalf, D'Agostimo and Hunter, 1971) identified as closed circle DNA (Parsons, Karol and Simpson, 1968). Uptake of ^{3}H-TTP has also been reported in isolated mitochondria of *Neurospora* (Koke, Malhotra and Bryan, 1970). However, incorporation into the protozoan *Urospora* conceivably could result from symbiotic or ingested microorganisms.

The obvious lack of cytoplasmic uptake of either ^{3}H-TdR or ^{3}H-TTP into the growing oocytes of *Pectinaria* implies that biosynthsis of mitochondrial DNA has also been completed. Oocytes typically possess many mitochondria. Mature oocytes of the amphibian egg contain 10^5 times as many mitochondria as somatic cells (Chase and Dawid, 1972). The amount of DNA in the unfertilized sea urchin egg is estimated as seven times the haploid amount of which 80–90% is mitochondrial DNA (Piko, Tyler and Vinograd, 1967). Isolated mitochondria from unfertilized mature eggs of the Loach showed little uptake of ^{3}H-TTP but induction of breaks in the mitochondrial DNA with an antibiotic, bruneomycin, could stimulate incorporation (Gause and Mikhailov, 1973).

Even though the growing oocytes in the vegetative phase showed no indication of taking up ^{3}H-TTP, the nuclear uptake in the preoocytes of the ovary repeated that found earlier with ^{3}H-TdR (Tweedell, 1966). Exposure of the ovary to ^{3}H-TTP also resulted in intense labeling over random parts of clumps of pre-

FIGURE 8. A free swimming blastula exposed to ^{3}H-TTP plus an excess of unlabeled thymidine for 1.5 hours. Several nuclei (arrows) were still heavily labeled with no apparent reduction in label; scale is 18 microns.

oocytes. These cells, labeled in the S-period prior to oocyte maturation in the G_2 interval, were resistant to DNAase treatment. This characteristic of nonextractibility is indicative of replictaing DNA (Friedman and Mueller, 1968).

Fertilized eggs and developing embryos of *Pectinaria* (2 cell through the gastrulae) all showed vigorous nuclear labeling after pulsing with ^{3}H-TTP that was indistinguishable from that obtained with ^{3}H-TdR. Cells pulsed at the 2 cell stage could be traced, with minimal label dilution, into the gastrula stage. Direct utilization of thymidine triphosphate into intact nuclei of living cells *in vivo* has been reported rarely, although nuclear uptake of ^{3}H-dTTP was indicated in 18 hr growing *Stentor* cells (de Terra, 1967). Likewise, changing the culture conditions to make cells "leaky" (Buttin and Kornberg, 1966) or more permeable to nucleotides (Moses and Richardson, 1970) has permitted uptake of TTP into cells of *E. coli*.

A similar correlation might then be advanced that *in vivo* use of TTP by embryonic cells is somewhat unique. Thus far, the reported observations have been limited to free-living single cell systems (*E. coli* and *Stentor*). The experiments on developing embryos of *Pectinaria* suggest that the labeled nucleotide, ^{3}H-TTP is incorporated into the nuclear DNA of all stages from fertilization through the gastrula stage.

There are numerous examples of the direct use of TTP by isolated nuclei. Under appropriate *in vitro* conditions isolated nuclei of either HeLa cells (Friedman and Mueller, 1968), rat thymus (Lagunoff, 1969), and rat liver (Lynch *et al.*, 1970; Cook, 1972) are able to incorporate thymidine triphosphate. Similarly, the herterochromatic fractions from mouse liver nuclei incorporate TTP into DNA (Klose and Flickinger, 1972).

The *in vitro* studies do suggest there is a strong difference in the degree of TTP utilization between normal nuclei and those obtained from fetal, regenerating or neoplastic tissues. Lynch *et al.* (1970) reported that isolated liver nuclei from regenerating liver incorporate ^{3}H-TTP at 10 times the rate of normal liver nuclei. However, Kaufman *et al.* (1972) found that while both normal and regenerating liver nuclei were active *in vitro*, normal nuclei are inactive *in vivo*, the latter reflecting unscheduled DNA synthesis.

When DNA synthesis of isolated liver nuclei is compared with strains of Morris hepatoma nuclei, the latter utilized ^{3}H-TTP from six to ten times more *in vitro* and exceeded parallel incorporation of ^{3}H-TdR *in vivo* (Ove, Coetzee and Morris, 1971). The uptake of ^{3}H-dTTP into DNA of nuclei isolated from Ehrlich ascites tumor cells was found to increase in proportion to the amount of X-irradiation of cells *in vivo* (Matsudaira and Furundo, 1971). Likewise, the antitumor antibiotic phleomycin will also stimulate incorporation of TTP into nuclei isolated from HeLa cells, osteosarcoma cells and transformed cells (Friedman, Stern and Rose, 1974). It is relevant that isolated rat liver mitochondria which incorporate dTTP or dATP *in vitro* (Kalf *et al.*, 1971) are also stimulated by cytoplasmic factors present in the postmicrosomal fraction of rat and mouse tumors and in regenerating and fetal rat liver, but they are absent from normal adult liver.

Entrance of the nucleotide macromolecules into intact developing cells is an unlikely problem. There is good evidence that DNA can penetrate mammalian or other cells in tissue culture (Ledoux, 1965; Robins and Taylor, 1968; Hill and

Hillova, 1971). Exogenous high molecular weight DNA (HMW ^{3}H-DNA) can even enter cleaving mouse embryos *in vitro* more proficiently than ^{3}H-TdR (Snow and McLaren, 1974).

Another difference and one of the basic requirements reported in most of the previous investigations for uptake of TTP into leaky cells or subcellular fractions has been the dependence on the presence of all four deoxyribonucleotides for utilization of labeled dTTP. The addition of the 3 nucleoside triphosphates seemed to have little effect on labeling of nuclei in early embryos of *Pectinaria* after exposure to ^{3}H-TTP.

The direct utilization of ^{3}H-TTP might be interpreted as a result of the dephosphorylation of ^{3}H-TTP to ^{3}H-TdR with its subsequent incorporation into DNA of the nucleus. This would either presume the release of phosphatases from the cell or dephosphorylation of TTP after cell entry. The application of cold thymidine 550 times in excess of ^{3}H-dTTP was an attempt to show that TTP was not being dephosphorylated before uptake. If TTP was being reduced to thymidine before uptake, the cold thymidine should successfully compete with the radioactive thymidine and reduce the label but in fact there was no decrease. The lack of any apparent reduction in the nuclear label from ^{3}H-TTP mitigates against the conversion of TTP to TdR by exogenous phosphatases. If such conversion was taking place the unlabeled thymidine pool would be expected to dilute out the labeled thymidine so that less nuclear labeling occurred.

On the other hand, if there was not a conversion of ^{3}H-TTP to ^{3}H-TdR, then this implies that ^{3}H-TTP either has a competitive advantage over the unlabeled TdR or that it was inhibiting TdR. Such a possibility exists since the presence of TTP has a feedback inhibition on the thymidine kinase in mammalian cells (Breitman, 1965; Kit, Dubbs and Frearson, 1964) and in *Xenopus* ooctyes (Woodland, 1969). The effect seems to be specific for dTTP (Ives, Morse and Potter, 1963) as opposed to other nucleotides.

Summary

1. Exposure of developing *Pectinaria* embryos to ^{3}H-TTP results in immediate nuclear label over all postfertilization stages.

2. Intense nuclear label also occurs over preoocytes or oogonia within the ovary after *in vivo* ^{3}H-TTP pulsing.

3. No nuclear uptake is obtained in either the packet or solitary vegetative oocyte with either ^{3}H-TTP or ^{3}H-TdR until after fertilization. There was no detection of mitochondrial DNA synthesis in oocytes but cytoplasmic labeling of a gregarine protozoan occurred *in vivo* with ^{3}H-TTP.

4. The addition of complementary nucleosides or nucleotides has no effect on the quantitative uptake of either radioactive precursor into oocytes or developing embryos.

5. These experiments suggest that ^{3}H-TTP is being utilized by dividing cells of *Pectinaria* embryos. Actual incorporation of ^{3}H-TTP rather than degradation of TTP to TdR was implied from observations showing that no reduction of nuclear ^{3}H-TTP occurred in the presence of excess unlabeled thymidine.

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